09937.864

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
14014.0319P1		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US 00/ 08588	31/03/2000	01/04/1999
Applicant		
THE GOVERNMENT OF THE UNITED STATES OF AMERICA, as		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

[ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

[ ] contained in the international application in written form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readble form.

[ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

[X] the text is approved as submitted by the applicant.

[ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

[X] the text is approved as submitted by the applicant.

[ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 1

[X] as suggested by the applicant.

[ ] None of the figures.

[ ] because the applicant failed to suggest a figure.

[ ] because this figure better characterizes the invention.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/08588

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/68 //G01N33/50

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12Q G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 38313 A (PARTIN ALAN W ;TS O PAUL O P (US); LESKO STEPHEN A (US); WANG ZHEN) 16 October 1997 (1997-10-16) page 20, line 22 -page 26, line 5 ---	1-15,17, 19,20
X	WO 97 46702 A (UNIV CALIFORNIA) 11 December 1997 (1997-12-11) claims 1-19 ---	11-15, 17,19,20

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 September 2001	26/09/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Osborne, H

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	HESELMEYER-HADDAD K ET AL: "Interphase cytogenetics with tumor-stage specific probes for the detection of tumor progression in cervical and mammary carcinogenesis" PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH , vol. 40, March 1999 (1999-03), page 537 XP001024240 see Abstract 3538; also Abstracts 3539-43. ---	1-15,17, 19,20
X	GHADMI B ET AL: "Specific chromosomal abberations and amplification of the AIB1 nuclear receptor coactivator gene in pancreatic carcinomas" AMERICAN JOURNAL OF PATHOLOGY, vol. 154, no. 2, February 1999 (1999-02), pages 525-36, XP001024303 see abstract ---	1-15,17, 19,20
X	HEYSELMEYER K ET AL: "advanced-stage cervical cancer are defined by a reurrent pattern of chromosomal abberations revealing high genetic instability and a consistent gain of chromosome arm 3q" GENES, CHROMOSOMES AND CANCERTIGATIONS, vol. 19, no. 4, 1997, pages 233-40, XP001024299 the whole document ---	1-21
X	WO 96 02002 A (SCHALKEN JACK A ;DEBRUYNE FRANS M J (NL)) 25 January 1996 (1996-01-25) page 5, line 5 -page 10, line 17 ---	16,18,21
X	RACILA E ET AL: "Detection and charcterization of carcinoma cells in the blood" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE. WASHINGTON, US, vol. 95, April 1998 (1998-04), pages 4589-4594, XP002132943 ISSN: 0027-8424 see "Abstract" and "Discussion" ---	16,18,21
A	MAKAROVSKIY A N ET AL: "APPLICATION OF IMMUNOMAGNETIC BEADS IN COMBINATION WITH RT-PCR FOR THE DETECTION OF CIRCULATING PROSTATE CANCER CELLS" JOURNAL OF CLINICAL LABORATORY ANALYSIS, NEW YORK, NY, US, vol. 11, 1997, pages 346-350, XP000872241 the whole document -----	1,3,6

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 00/08588

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9738313	A	16-10-1997	AU	2438497 A	29-10-1997
			CA	2251186 A1	16-10-1997
			CN	1221492 A	30-06-1999
			EP	0891550 A1	20-01-1999
			JP	2000508171 T	04-07-2000
			WO	9738313 A1	16-10-1997
			US	5962237 A	05-10-1999
WO 9746702	A	11-12-1997	US	5925519 A	20-07-1999
			EP	0954607 A1	10-11-1999
			JP	2000511433 T	05-09-2000
			WO	9746702 A1	11-12-1997
WO 9602002	A	25-01-1996	AU	2897195 A	09-02-1996
			WO	9602002 A1	25-01-1996

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark Office, PCT
2011 South Clark Place Room CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 29 November 2000 (29.11.00)	
International application No. PCT/US00/08588	**Applicant's or agent's file reference** 14014.0319P1
International filing date (day/month/year) 31 March 2000 (31.03.00)	**Priority date** (day/month/year) 01 April 1999 (01.04.99)
Applicant RIED, Thomas et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

31 October 2000 (31.10.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer R. Forax
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

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PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
14014.0319P1		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US00/08588	31/03/2000	01/04/1999
International Patent Classification (IPC) or national classification and IPC C12Q1/68		
Applicant THE GOVERNMENT OF THE UNITED STATES OF AMERICA et		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
31/10/2000	10.10.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Knudsen, H Telephone No. +49 89 2399 8696

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US00/08588

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-36 as originally filed

Claims, No.:

1-21 as originally filed

Drawings, sheets:

1/4-4/4 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:
see separate sheet

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 1-18,20-21 (IA).

because:

☒ the said international application, or the said claims Nos. 1-18,20-21 (IA) relate to the following subject matter which does not require an international preliminary examination (*specify*):
see separate sheet

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

☐ restricted the claims.

☐ paid additional fees.

☐ paid additional fees under protest.

☐ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☒ not complied with for the following reasons:
see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☒ all parts.

☐ the parts relating to claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	7,14-15,17,20
	No:	Claims	1-6,8-13,16,18,21
Inventive step (IS)	Yes:	Claims	
	No:	Claims	7,14-15,17,20
Industrial applicability (IA)	Yes:	Claims	
	No:	Claims	

2. Citations and explanations
see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

Re Item I

Basis of the opinion

The present set of claims do not contain a claim no. 19.

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The expressions "obtaining a cell" and "obtaining a biological sample", respectively, are considered an in-vivo treatment and claims 1-18 and 20-21 therefore relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

Re Item IV

Lack of unity of invention

In claims 1-15, 17 and 20, the cancer cells are determined by the detection of a hybridisation pattern. The method of claims 16, 18 and 21 employ the detection of complex formation. It appears that these two groups of claims are not linked by a common inventive concept in view of the cited prior art documents.

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive st p or industrial applicability; citations and explanations supporting such statement

Reference is made to the following document/s/:

D1: WO 97/38313 (PARTIN ALAN W ;TS O PAUL O P (US); LESKO STEPHEN A (US); WANG ZHEN) 16 October 1997

D2: WO 97/46702 (UNIV CALIFORNIA) 11 December 1997

D3: HESELMEYER-HADDAD K ET AL: 'Interphase cytogenetics with tumor-stage specific probes for the detection of tumor progression in cervical and mammary carcinogenesis' PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH , vol. 40, March 1999, page 537

D4: HEYSELMEYER K ET AL: 'Advanced-stage cervical cancer are defined by a recurrent pattern of chromosomal aberrations revealing high genetic instability and a consistent gain of chromosome arm 3q' GENES, CHROMOSOMES AND CANCER, vol. 19, no. 4, 1997, pages 233-40

D5: RACILA E ET AL: 'Detection and characterization of carcinoma cells in the blood' PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE. WASHINGTON, US, vol. 95, April 1998, pages 4589-4594, ISSN: 0027-8424

NOVELTY:

D1 discloses the enrichment of rare, eg prostate cancer, cells from a blood sample (see abstract) by immunomagnetic procedures and the subsequent identification of rare cells by hybridisation with fluorescently labelled probes (see page 23). Exemplified are probes against PSMA, PSA, centromeric regions of chromosomes 7, 8 and 18 (see pages 20-22) and the concurrent use of a plurality of probes (see Example 7). The hybridisation pattern may be read on a slide. Thus, D1 is novelty destroying for claims 1-6 and 8-13.

D2 discloses a method for detecting prostate cancer by detecting prostate cancer cells in body fluids. It is mentioned on page 14, line 28 that the sample used in FISH may be blood. The different embodiments mentioned in the claims of D2 show that D2 is novelty destroying for claims 2-5 and 8-13

D3 discloses an in-situ hybridisation method which involves the use of a 3q probe for detection of cervical carcinomas. D3 is therefore novelty destroying for claims 1-2 and 11-12.

D4 discloses a method with which advanced stage cervical carcinomas are detected by comparative genomic hybridisation (CGH). The DNA prepared from a karyotypically female donor represents a probe in CGH. Thus, claims 1-2 lack novelty over D4.

D5 discloses that levels of tumour cells in circulation correlates well with clinical status and treatment with chemotherapy (see abstract). The method used for detection of the tumours is labelling with anti-cytokeratin and anti-mucin-1 antibodies. The said antibodies form a complex with tumour cells and are thereafter detected. Thus, D5 is novelty destroying for claims 16, 18 and 21.

Summarising, claims 1-6, 8-13, 16, 18 and 21 lack novelty over the cited prior art documents.

INVENTIVE STEP:

As explained in D1, the conventional method of enriching samples containing rare cells is the use of an antibody against a ligand present on the rare cells (see D1, sentence bridging pages 7 and 8). Since it is well-known that cytokeratin is expressed on prostate cancer cells, the use of anti-cytokeratin antibodies in the enrichment of prostate cancer cells from a blood sample would be obvious to the skilled person. Thus, claim 7 does not appear to be inventive.

Claim 14 differs from D1 only in that the sample is enriched by positive selection. Thus, claim 14 is not considered inventive for the same reasons as claim 7.

The closest prior art for claims 15, 17 and 20 is represented by D5. The difference between the subject-matter of the said claims and D5 resides in the use of a hybridisation probe and the subsequent detection of a hybridisation pattern for determining the number of cancer cells. The problem solved by the method of the said claims therefore is the provision of an alternative method for determining the amount of cells in circulation. D1 and D2 disclose that cancer cells taken from the circulation may be detected by probe hybridisation and the development of an alternative method based on probe hybridisation would therefore appear to be obvious to the skilled person. Thus, claims 15, 17 and 20 are not considered inventive.

INDUSTRIAL APPLICABILITY:

For the assessment of the present claims 1-18 and 20-21 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to a diagnostic method carried out on the living human or animal body.

Re Item VII

Certain defects in the international application

7.1 Contrary to the requirements of Rule 5(a)(ii) PCT, the closest prior art documents D1, D2 and D5 are not identified in the description and the relevant background art disclosed therein is not briefly discussed.

7.2 The vague and imprecise statement in the description on page 28, 2nd paragraph implies that the subject-matter for which protection is sought may be different to that defined by the claims, thereby resulting in lack of clarity (Article 6 PCT) when used to interpret them (see also the PCT Guidelines, PCT Guidelines C-III, 4.3a).

7.3 It is not possible to incorporate the teaching of a prior art document into the present application's disclosure by the expression "herein incorporated by reference" or equivalents thereof (see p.36, last paragraph) (cf PCT Guidelines, C-II, 4.17).

7.4 Contrary to the PCT Guidelines C-II 4.16-4.17, registered trade marks, eg "Tween 20", have not been identified as such in the description.

09193864

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
12 October 2000 (12.10.2000)

PCT

(10) International Publication Number
WO 00/60119 A3

(51) International Patent Classification[7]: C12Q 1/68
// G01N 33/50

(21) International Application Number: PCT/US00/08588

(22) International Filing Date: 31 March 2000 (31.03.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/127,367 1 April 1999 (01.04.1999) US

(71) Applicant *(for all designated States except US)*: **THE GOVERNMENT OF THE UNITED STATES OF AMERICA, as represented by THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES** [US/US]; National Institutes of Health, Office of Technology Transfer, Suite 325, 6011 Executive Boulevard, Rockville, MD 20852-3804 (US).

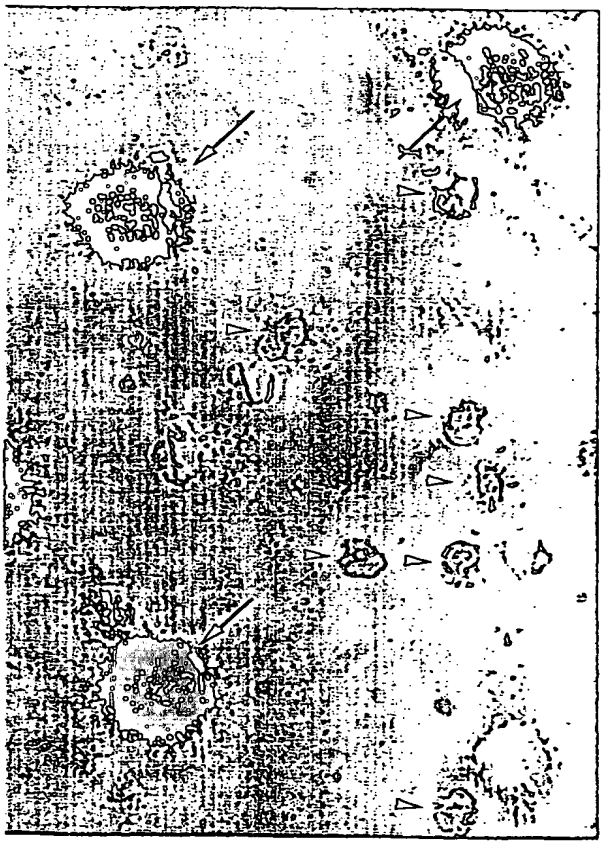
(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **RIED, Thomas** [DE/US]; 9819 Parkwood Drive, Bethesda, MD 20814 (US). **UHR, Jonathan** [US/US]; 12311 Shiremont Drive, Dallas, TX 75230 (US). **GHADIMI, Bijan, M.** [DE/US]; 4521 Windsor Lane, Bethesda, MD 20814 (US). **SCHROCK, Evelin** [DE/US]; 13004 Atlantic Avenue, Rockville, MD 20851 (US). **AUER, Gert** [DE/SE]; Wibomsvag 12, S-171 60 Solna (SE).

(74) Agents: **KERBER, Lori, L.** et al.; Needle & Rosenberg, P.C., Suite 1200, 127 Peachtree Street, N.E., Atlanta, GA 30303-1811 (US).

[Continued on next page]

(54) Title: METHODS FOR DETECTING CANCER CELLS



(57) Abstract: The invention relates to a highly sensitive assay for distinguishing between cancer and non-cancer epithelial cells in the blood that provides an improved diagnostic technique for detecting cancer and determining the organ-origin of the cancer.

WO 00/60119 A3

(81) **Designated States** *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) **Designated States** *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report*

(88) **Date of publication of the international search report:**
10 January 2002

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

1: J Immunol Methods 1995 Jun 28;183(2):251-65 Related Articles, Links

ELSEVIER SCIENCE FULL-TEXT ARTICLE

An immunological enrichment method for epithelial cells from peripheral blood.

Griwatz C, Brandt B, Assmann G, Zanker KS.

Institut fur Immunologie, Naturwissenschaftliche Fakultat der Universitat Witten/Herdecke, Germany.

The ability of primary tumours to metastasize accounts for the majority of cancer deaths. The emergence of circulating carcinoma cells in the peripheral blood is supposed to be an indicator for cancer cell spread. We have focused on this phenomenon in order to develop a sensitive technique for enriching epithelial derived cells on the basis of a two-layer density gradient and subsequent immune magnetic cell sorting. Epithelial cells are possess a cytoskeleton containing an assembly of intermediate filaments. During carcinogenesis these filaments do not undergo modifications of antibody binding epitopes such as occur in the protein domains of surface markers. We have developed a two-layer density gradient in which the epithelial cells form a single density band. This was demonstrated by recovery experiments using [3H] thymidine-labelled epithelial cells which showed epithelial cells were enriched within this first step by a factor of 20. In a second step the MACS system was applied. Cells were stained with a performed FITC-conjugated mouse anti-human cytokeratin antibody bound to a rat anti-mouse antibody coupled to superparamagnetic particles (immune paramagnetic separation complex; IPSC) and subjected to high gradient magnetic fields. The two-step procedure was confirmed by dispersing 50 epithelial cells in 5 x 10(5), 5 x 10(6), 5 x 10(7), 5 x 10(8), 5 x 10(9) peripheral blood leucocytes. Specific binding of the preformed IPSC was demonstrated by flow cytometry, confocal laser, fluorescent and electron microscopy. The specificity of the method was further proved by dual

staining with IPSC and anti-human PSA antibody of epithelial prostatic cells separated from peripheral blood in vitro. By means of this double-step separation method it was possible to isolate up to 15-20 cells out of 50 epithelial cells originally suspended into 5 x 10(7) to 5 x 10(9) human peripheral blood leucocytes. This represented an enrichment factor between 20,000 and 200,000, depending on the initial cell number. The immunologically captured epithelial cells can be used for further cytogenetic investigations such as in situ hybridization (ISH) and/or polymerase chain reaction (PCR) to detect cancer cell specific gene aberrations. This sensitive combined buoyant density immune magnetic cell separation technique is capable of detecting free carcinoma cells in the peripheral blood.

PMID: 7602148 [PubMed - indexed for MEDLINE]
